Plasmonic nanoparticles decorated with fluorescently-labelled mRNA: using single-pair FRET effect for investigating enzymes active in mRNA metabolism

Fluorescence Resonance Energy Transfer (FRET) is one of the most fundamental processes at the nanoscale. In the classical description, when two emitters are placed at distances of approximately 10 nm, the energy can be transferred between them in a non-radiative way. In order for this process to occur, the emission of one molecule (donor) has to overlap spectrally with the absorption of the second molecule (acceptor). Also, the dipole moments of the molecules should be arranged properly. Spectroscopically, the FRET process is recognized as shortening of the fluorescence decay time of the donor and/or increase of the fluorescence intensity of the acceptor.

Since the efficiency of FRET depends on the inverse-sixth power of the distance between donor and acceptor, the concept of using the energy transfer has been swiftly implemented in biology, biochemistry, and biophysics for measuring distances and detecting molecular interactions. In a typical experiment aimed at resolving protein dynamics, a protein is labelled with two dyes, which feature energy transfer of a given efficiency at the folded state. Upon denaturation, unfolding or any conformational change of the protein, the distance between donor and acceptor also changes leading to variation of the energy transfer, frequently measured as an increase of the fluorescence intensity of the donor. Another strategy is to use the FRET process for sensing by designing a probe towards the required activity, for instance detection of the presence of an enzyme. In this case, a DNA chain or a peptide sequence, connects donor and acceptor molecules, and at the same time contains a segment sensitive to the enzyme. In a neutral configuration, the energy transfer between donor and acceptor takes place, however, upon introducing the enzyme, the chain connecting the donor and acceptor breaks and FRET vanishes.

The aim envisioned in this interdisciplinary project is experimental optical detection and quantification of the activity of the enzymes responsible for mRNA degradation using novel double-labelled nucleotide- and mRNA-based fluorescent probes, whose optical response can be strongly enhanced with plasmon excitations in silver nanowires. To this end we want to synthesize appropriate probes, conjugate them specifically with silver nanowires and using advanced fluorescence imaging, monitoring in real-time changes of the FRET efficiency upon incorporation of particular enzymes.

Particular attention will be placed at using double-labelled RNA FRET probes, as they provide a way to monitor various RNA hydrolases. The ability to monitor enzymes with RNA is important for regulating the expression of genetic information. Fluorescent labelling of RNA plays crucial role in developmental and structural studies, as well as investigations of gene expression, cellular immune responses, and delivery of mRNA based therapeutics, thus creating the drive to improve and expand the current toolbox of chemo-enzymatic RNA modifications.